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Fluorescence polarization in gastric aspirate and in amniotic fluid and its relationship to the respiratory distress syndrome

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1 Introduction

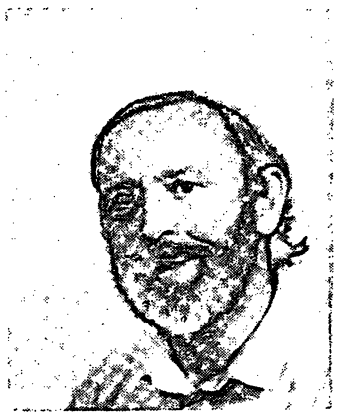
There are many tests to predict fetal lung maturity after amniotic fluid analyses [1]. A rather new test is the determination of fluorescence polarization of 1,6-diphenyl-1,3,5-hexatriene (DPH), added to amniotic fluid [2]. The probe DPH interacts with the lipid molecules and thus also with surfactant material, secreted by the fetal lung in utero. The value of fluorescence polarization for prediction of the development of the respiratory distress syndrome (RDS) is good. According to BLUMENFELD et al. [3], it is even better than that of the lecithin/sphingomyelin ratio.

Several tests, which are used for the estimation of fetal lung maturity in amniotic fluid, have been carried out also on tracheal [4], pharyngeal [4, 5], or gastric aspirates [6, 7] of the newborn. Nevertheless, data on the relationship between these tests and neonatal respiratory function are conflicting. So far, good results were obtained by estimating the fatty acid composition of lecithins, isolated from the gastric aspirate of the newborn [7]. However, the methodology is complex, time consuming and therefore not very useful for routine purposes.

The measurement of fluorescence polarization of DPH is a rapid and easy technique [2], but only recently fluorescence polarization has been studied in aspirates of newborns [8, 9]. CASPI et al. [8] found a 100% predictability whether RDS would develop. The results of LUERTI and coworkers [9] however, showing a less significant relationship between the fluorescence polarization of gastric aspirate and gestational age than between the fluorescence polarization of amniotic fluid and fetal age, might indicate that the gastric aspirate values are less reliable in predicting the risk of RDS.

We here report our findings of fluorescence polarization in gastric aspirates, correlate these results with neonatal lung function and conclude that fluorescence polarization values of gastric aspirates are unreliable to predict neonatal lung (im)maturity.

Curriculum vitae



DR. HANS EGBERTS *was born in 1943 in Aalten, the Netherlands. He studied veterinary science at the State University of Utrecht from which he graduated in 1970.*
He upheld his Ph. D. thesis entitled: Prenatal lung maturation and hyaline membrane disease in lambs in 1977. Since 1976 he has worked as a scientist at the Department of Obstetrics & Gynecology of the University Hospital in Leiden. His principal interest is: Surfactant.

Materials and methods

Gastric aspirates were obtained immediately after delivery from 51 infants. Of these, 23 were born at 26–36 weeks of gestation and 28 at 37 weeks or more. Both amniotic fluid and gastric aspirate were obtained in thirteen cases. All samples were kept at −20 °C until analysis.

Fluorescence polarization was measured by means of a Perkin Elmer 650-10M fluorescence spectrofluorometer with polarization attachment. After thawing, the sample was mixed and centrifuged for 10 minutes at 150 x g. Thereafter, 0.5 mL of the supernatant was mixed with 3.5 mL of a solution of DPH (KOCH-LIGHT, Colnbrook, U.K.) in phosphate buffered saline (PBS). This DPH solution was prepared freshly by mixing 200 μL of a stock solution (2 mmol/L in tetrahydrofuran) with 1 L of PBS. The gastric aspirate or amniotic fluid, mixed with the DPH-PBS solution, was incubated for 30 minutes at 37 °C and measured at 22 °C in triplicate. The fluorescence polarization (FP) value was computed according to the formula:

$$FP = (I_{\parallel} - I_{\perp})/(I_{\parallel} + I_{\perp})^{*}.$$

According to their clinical pulmonary condition, infants were divided in four groups. In group I (n = 4), three infants had RDS and another infant of 26 weeks gestation, died from pulmonary immaturity within 24 hours. Group II (n = 4) consists of preterm infants with transient pulmonary problems (birth asphyxia, amniotic fluid/meconium aspiration). Group III (n = 15) consists of preterm infants without respiratory problems. Group IV (n = 28) included term (37 weeks or more) infants with normal lung function.

Respiratory distress syndrome (RDS) was diagnosed if all of the following criteria were present: (1) respiratory rate $> 60\ min^{-1}$ for more than 24 hours, (2) SILVERMAN score > 2 for more than 24 hours, (3) cyanosis in air breathing and (4) chest X ray RDS stages.

$I_{\parallel}$ = fluorescence intensity with parallel emission and excitation polarizers.

$I_{\perp}$ = fluorescence intensity with emission and excitation polarizers perpendicular.

Results

The fluorescence polarization value FP of gastric aspirates of preterm infants (mean ± SD = 0.322 ± 0.043; n = 23) was significantly higher ($P < 0.05$) than those of term infants (0.298 ± 0.034; n = 28). A low but significant negative correlation ($r = -0.32$; $P < 0.05$) was found between gastric aspirate FP values and gestational age. The range of values in preterm infants (0.229 – 0.378) was similar to that in term infants (0.224 – 0.359). Fig. 1 shows that in gastric aspirate, low FP values were found more often before the 36th week of gestation, than in amniotic fluids ($P < 0.05$). Exponential regression showed a highly significant negative correlation between the fluorescence polarization values of amniotic fluid and gestational age ($r = -0.88$; $P < 0.01$) and all of these values were within the range of amniotic fluid FP values, estimated previously (Fig. 1).

Tab. I shows fluorescence polarization values for infants with respiratory problems and for infants in whom gastric aspirate values could be correlated to amniotic fluid values. There was no relationship

Tab. I. Fluorescence polarization of gastric aspirates and amniotic fluids of newborns with or without Respiratory Distress Syndrome.

Group	Gestational age (wks)	FP value gastric aspirate	FP value amniotic fluid
I	26*	0.366	—
Respiratory	31	0.281	0.341
Distress	31	0.347	0.338
	33	0.304	—
II	29	0.303	—
Respiratory	31	0.343	0.324
Adaptation	32	0.229	0.318
	34	0.322	—
III	33	0.329	0.315
Preterm	34	0.264	0.278
Normal	35	0.341	0.293
	35	0.333	0.319
	35	0.289	0.298
	36	0.328	0.293
IV	37	0.328	0.273
Term	39	0.279	0.239
Normal	40	0.309	0.283

* pulmonary immaturity; see materials and methods.

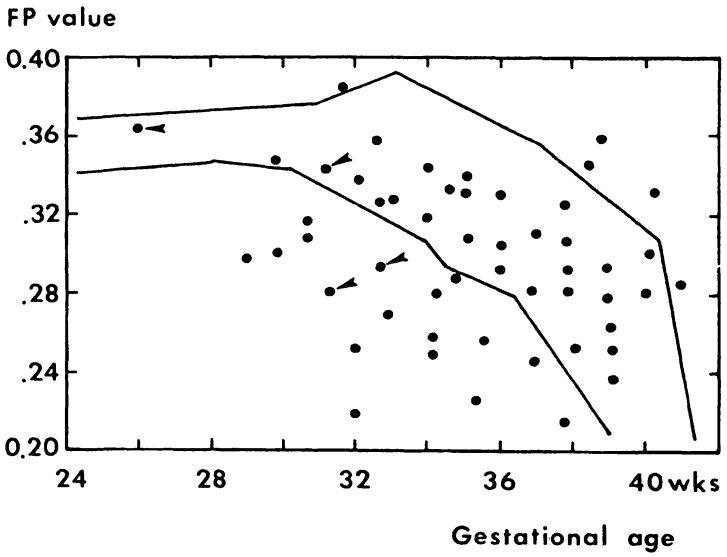


Fig. 1. Fluorescence polarization values in gastric aspirates of newborns. The area between the lines represents the range of FP values in amniotic fluids, which were estimated previously (n = 86).
The values of infants with RDS are marked by an arrow.

between the FP values of gastric aspirates and those of amniotic fluids ($r = 0.23$; $P > 0.1$). The highest FP values in amniotic fluid (> 0.337) were found in the two samples of preterm infants with RDS. Respiratory distress was not observed at amniotic fluid FP values of 0.324 or less. On the other hand fluorescence polarization values in gastric aspirates showed no relationship with the occurrence of RDS. Both high and low FP values were found in aspirates of preterm and term neonates.

Discussion

Fluorescence polarization of DPH changes in amniotic fluid as well as in gastric aspirate with gestational age. The fluorescence polarization value in amniotic fluid decreases strikingly during pregnancy, whereas gastric aspirate values decline only slightly. The changes in the FP values of amniotic fluid are in accordance with the findings of others [2, 3], which implies that fluorescence polarization can adequately be measured by means of a Perkin Elmer spectrofluorometer and its polarization adaptors. The number of fluorescence polarization measurements in amniotic fluid and the number of RDS cases are too small to determine a cut-off value, below which RDS will not occur. We noted, however, that the FP values of infants with RDS were above most of the cut-off values, reviewed by CHESKIN and BLUMENFELD [2]. The mean FP value in gastric aspirates seems to be lower than in amniotic fluids. This is probably because of direct ingestion of surfactant material that is present in the laryngopharynx. Therefore, the cut-off value to predict RDS will be lower in gastric aspirate than in amniotic fluid, which is indicated by CASPI et al. [8]. Our results, however, which are approximately the same as those of LUERTI et al. [9], would than lead to many false positive and false negative predictions. We can not explain the discrepancy between our results and those of CASPI et al. [8], who found a 100% predictivity at a cut-off value of 0.320.

The discrepancy in findings between the predictive value of fluorescence polarization in amniotic fluid and gastric aspirate may find its origin in the presence of interfering substances. Whole blood, serum or meconium affects the FP value in amniotic fluid [3]. The samples of infants in whom RDS developed, however, were not contaminated. Furthermore, the correlation between the fluorescence polarization value in gastric aspirate and gestational age did not improve after discarding contaminated samples. It was previously found in our laboratory [10] that slime and flocculent material, present in gastric aspirates, attracts surfactant. Therefore, interaction between slime and surfactant may affect the fluorescence of the DPH-phospholipid complexes. Sofar, our data indicate that the measurement of fluorescence polarization in gastric aspirate is not a useful procedure. A low value does not predict correctly whether RDS will be absent in the preterm infant, whereas high values can be found in term babies without respiratory problems.

Some form of pretreatment of the gastric aspirate may increase the predictive value of its fluorescence polarization results. It may therefore be better to estimate the FP value of amniotic fluid, obtained by amnioscopy, for the assessment of perinatal lung maturity.

Summary

Fluorescence polarization of diphenylhexatriene (DPH), added to amniotic fluid can be used to estimate fetal lung maturity. In this study we estimated the fluorescence polarization (FP) value of gastric aspirates of newborns and of amniotic fluids and studied the relationship between the FP value and neonatal lung function.
Gastric aspirates of 51 newborns and amniotic fluids of 13 cases were mixed with a DPH-phosphate buffered saline solution and incubated for 30 minutes at 37 °C. Fluorescence polarization was measured at 22 °C.
The FP values of amniotic fluid are in accordance with results previously found. A highly significant negative correlation was present between the FP value in amniotic fluid and gestational age. The highest FP values were found in amniotic fluids of infants in whom respiratory distress developed.
The FP values of gastric aspirates decreased also with gestational age. A rather poor negative correlation, however, was found between these FP values and gestational age. We also noticed that, before the 36th week of gestation, the FP values in gastric aspirates tend to be lower than those in amniotic fluids.
Both high and low FP values were estimated in gastric aspirates of infants with RDS and of infants without respiratory problems. These results show that the measurement of the FP value in gastric aspirates of newborns is not an useful method to determine whether the infant will develop RDS or not.

Keywords: Amniotic fluid, fetus, fluorescence polarization, gastric aspirate, lung maturity, newborn, respiratory distress.

Zusammenfassung

Fluoreszenzpolarisation im Magensaft und im Fruchtwasser und ihre Aussagekraft bezüglich eines RDS.
Die Fluoreszenzpolarisation (FP) von dem Fruchtwasser zugesetztem Diphenylhexatrien (DPH) kann als Parameter zur Einschätzung der fetalen Lungenreife herangezogen werden. In unserer Studie bestimmten wir die FP-Werte im aspirierten Magensaft Neugeborener sowie im Fruchtwasser und untersuchten die Beziehungn zwischen den FP-Werten und neonataler Lungenfunktion.
Magensaftproben von 51 Neugeborenen und Fruchtwasserproben von 13 Patientinnen wurden mit einer phosphatgepufferten DPH-haltigen Salzlösung gemischt und für 30 min bei 37 °C inkubiert. Die Fluoreszenzpolarisation wurde bei 22 °C gemessen.
Bezüglich der FP-Werte im Fruchtwasser stimmten unsere Ergebnisse mit denen früherer Untersuchungen überein. So fand sich eine hochsignifikante negative Korrelation zwischen den FP-Werten im Fruchtwasser und dem Gestationsalter. Die höchsten FP-Werte fanden sich in Fruchtwasserproben von Kindern, die ein RDS entwickelten.
Die FP-Werte im Magensaft sanken ebenfalls mit zunehmendem Gestationsalter ab. Es bestand jedoch nur eine angedeutete negative Korrelation zwischen diesen FP-Werten und dem Gestationsalter. Außerdem scheinen vor der 36. Schwangerschaftswoche die FP-Werte im Magensaft niedriger als im Fruchtwasser zu sein.
Wir fanden sowohl hohe als auch niedrige FP-Werte im Magensaft Neugeborener, die ein RDS entwickelten wie auch bei denen, die keine Respirationsstörungen hatten. Diese Ergebnisse zeigen, daß die FP-Werte im Magensaft Neugeborener nicht als prognostischer Parameter bezüglich der Entwicklung eines RDS verwertet werden können.

Schlüsselwörter: Fetus, Fluoreszenzpolarisation, Fruchtwasser, Lungenreife, Magensaft, Neugeborenes, Respirationsstörung.

Résumé

Polarisation en fluorescence du liquide d'aspiration gastrique et du liquide amniotique – relation avec le syndrome de détresse respiratoire
On peut utiliser la polarisation en fluorescence du diphenylhexatriene (DPH) ajouté au liquide amniotique pour estimer la maturité pulmonaire fœtale. Dans cette étude, nous avons déterminé la valeur de la polarisation en fluorescence (PF) du liquide d'aspiration gastrique des nouveaux-nés et du liquide amniotique; nous avons aussi étudié la relation entre la valeur de la PF et la fonction pulmonaire néonatale.
Les liquides d'aspiration gastrique de 51 nouveaux-nés et 13 liquides amniotiques ont été mélangés avec une solution saline tampon DPH-phosphate et incubés à 37 °C pendant 30 minutes. La polarisation par fluorescence a été mesurée à 22 °C.
Les valeurs de la PF du liquide amniotique concordent avec les résultats antérieurement trouvés. Une corrélation négative hautement significative existe entre la valeur de la PF du liquide amniotique et l'âge gestationnel. Les valeurs les plus élevées de la PF ont été trouvées dans les liquides amniotiques des enfants ayant développé une détresse respiratoire.
Les valeurs de la PF des liquides d'aspiration gastrique diminuent également avec l'âge gestationnel. Une corrélation négative assez faible a cependant été trouvée entre ces valeurs de la PF et l'âge gestationnel. Nous avons également remarqué qu'avant la 36ème semaine de

gestation, les valeurs de la PF dans les liquides d'aspiration gastrique tendent à être plus basses que celles de liquides amniotiques.
Chez les enfants avec SDR et chez les enfants sans problème respiratoire on a trouvé dans les liquides d'aspiration gastrique des valeurs de PF à la fois hautes et à la fois basses. Ces résultats montrent que la détermination de la valeur de la PF dans les liquides d'aspiration gastrique des nouveaux-nés n'est pas une méthode utile pour déterminer si les enfants développeront ou non un SDR.

Mots-clés: Détresse respiratoire, fœtus, liquide amniotique, liquide d'aspiration, maturité pulmonaires, nouveau-né, polarisation en fluorescence.

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